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Microbial Chitinases Purification: Conventional Protocols and Affinity Based Strategies (Review)

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Abstract: Microbial chitinases (EC.3.2.1.14) occupy a place of prominence among biocatalysts in nowadays biotechnology and have received tremendous interest among scientists and industrialists due to their wider range of biotechnological applications. Chitinases have been produced and purified to homogeneity from a large number of organisms ranging from bacteria and fungi to plant, animal and human; however the microbes still the major and the most industrially important source. In order to obtain chitinases in pure form for academic and biotechnological purposes, several purification protocols were proposed, this protocols varies in steps number, time and sequence, Furthermore they were laborious and cost and time consuming, for this raisons, The choice of the best purification protocol increasing the yields and folds still crucial and much recommended for enzyme characterization which help for understanding the biochemical aspects of microbial chitinases, in other hand the developing of an efficient large scale purification strategy remains a challenge to meet with increased industrial demands. The present review summarize the different purification protocols even conventional or affinity based, which have been employed for microbial chitinases and list the purification steps of each protocol comparing between them on basis of specific activity, yield and purification folds. As well as the recent available purification technologies also were reported. This review serves as an excellent literature reference and immense help for those working on microbial chitinases and planning for their purification.

Key words: Microbial Chitinase · Purification · Protocol · Chromatography · Affinity · Yield · Fold

INTRODUCTION

Chitinases (EC 3.2.1.14) are glycosyl hydrolases present in a wide range of prokaryotic and eukaryotic organisms, but industrially are produced by a number of microorganisms, including bacteria, actinomycetes, yeasts and fungi [1-4]. These enzymes are classified according to Henrissat and Bairoch [5] into subfamilies 18 and 19 on the basis of amino acid similarities within the catalytic domain.

Due to the expending applications of chitinases in various fields, it is important to understand their nature and properties for efficient and effective usage. In the last decades, vigorous research has been carried out on chitinases purification using different protocols varies in steps number, time and sequence, but the majority of them based on series of chromatography-based purification steps including ion exchange and size exclusion chromatographies. Recently much attention has been focused on chitin affinity chromatography for chitinases purification [6, 7] due to their rapidity, simplicity and higher selectivity, capacity compared to the conventional protocols.

This review recapitulates the different protocols mostly cited in literatures concerning microbial chitinases purification using even conventional or chitin affinity based methods.

Chitinases Conventional Purification: A number of researchers have successfully purified and characterized Chitinases from a variety of microorganisms using different protocols. The crude extract subjected to precipitation using ammonium sulfate fractionation then followed by a series of chromatography-based purification steps including ion exchange, size exclusion chromatography to separate proteins on the basis of

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Table 1: Chitinases Purification Using Conventional Protocols

Source / Reference	Purification steps	Yield (%)	Purification Fold
Acinetobacter sp. *strain CHB 101* /[8]	Culture supernatant, $(NH_4)_2$ SO_4 (0 – 80 %), CM-sepharose,	17	ND
	Sephadex G-100	16	ND
Aeromonas schubertii / [9]	Cell filtrate, Ammonium sulphate 90 %, IEF	10	2
Pseudomonas aeruginosa K-187 / [10]	Culture supernatant, $(NH_4)_2SO_4$ (80%),	27	10
	DEAE Sepharose CL-6B, $(NH_4)_2SO_4$ (80%), Econo Pac q	7	6
Cellulomonas flavigena NTOUI / [11]	Culture filtrate, Q cartridge chromatography,		
	hydrophobic interaction chromatography Superdex 75 HR	5.64	20.17
Enterobacter sp. *NRG4* / [12]	Cell free supernatant, $(NH4)_2SO_4$ (30 – 75 %), DEAE Sephadex,	31.1	44.12
	Sephadex G 200		
Serratia marcescens BJL200 / [13]	Diluted periplosmic extract, phenyl-superose HR 5/5 (FPLC)	79	ND
Vibrio alginolyticus H-8 /[14]	Culture filtrate, crude chitinase, 2nd DEAE-Toyopearl 650M,	10	8.3
	superdex 200 HR	6.7	14.5
*Xanthomonas sp. strain Ak *ch B* / [15]	Culture supernatant, (NH4)2SO4 100 %, phenyl Toyopearl 650 S,	NA	NA
	DEAE- Toyopearl 650M	53	16
Bacillus circulans WL-12 / [16]	Periplasmic fraction, $(NH4)_2$ SO_4 (40%),	90.6	33.7
	Q ceramic hyper D Ion exchange chromatography		
Bacillus stearothermophilus CH_4 / [17]	Culture filtrate, $(NH4)_2SO_4$ (80%), DEAE cellulose,	1.31	387
	Butyl-Toyopearl, Sephadex 6-100, Mono-Q		
Streptomyces thermoviolaceus OPC-520/ [18]	Culture filtrate, DEAE-Toyopeol 650 M, Sephadex G75,	29.9	20.6
	Phenyl Toyopearl 650 M, Mono-Q HR 5/5		
Streptomyces albovinaceus S-22 / [19]	Culture filtrate, Ammonium sulphate (80%), dialysis, Sephadex G200	40.07	2.3
Metarhizium anisopliae / [20]	Ammonium sulphate 85 %. DEAE Sephacel	2.74	2.5

ND: Not Determined

Table 2: chitinases purification using affinity based strategies

Source / Reference	Purification steps	Yield (%)	Purification Fold
Aeromonas caviae / [21]	Culture broth, H is Tag resin affinity.	1.1	3
	Cell extract, H is Tag resin affinity.	3.5	3
Serratia marcescens / [22]	Crude, colloidal chitin batch affinity, ultragel AC A54	ND	2 – 3
Bacillus sp. BG-11 / [23]	Cell free supernatant, $(NH4)_2SO_4$ (50 – 80%),	15	16
	affinity binding to chitin, Sephadex G-100		
Bacillus sp. strain MH-1 / [24]	Culture filtrate, chitin affinity (batch), chromate focusing	2.2	20
		0.67	18
		1.1	24
Paenibacillus sp. [25]	affinity adsorption on fermentation-processed chitin (FPC)	97	10.3
Streptomyces venezuelae P10[26]	80% Ammonium, sulfate precipitation, Affinity binding to chitin,	16.1	2.47
	DEAE-cellulose column		
Neurospora crassa / [27]	- Crude, affinity precipitation with 0.5 % (w/v) chitosan solution	85	27
	- Crude, PEG-chitosan salt two phase system	86	34
Rhizopus oligosporus / [28]	Culture filtrate, Ammonium sulphate 90 %, chitin affinity column,	1.8	13.3
	Sephadex G-75, DEAE-Toyopearl	1.7	6

charge and size respectively. Comprehensive information with respect to purification steps used, yield and fold of purified Chitinases were briefly summarized in Table 1.

Chitinases Chitin Affinity Purification: Procedures that utilize the affinities of biomolecules and ligands for enzymes purification are gaining increasing acceptance due to their selectivity and higher capacity compared to the conventional protocols. Concerning chitinases, chitin affinity chromatography, has been shown to be a promising approach for chitinase purification. Chitinases from various microbial sources were purified to near homogeneity using even batch or column affinity on chitin, colloidal chitin or chitosan with folds and yields reach to 34 and 86 % respectively [Table 2].

CONCLUSION

The choice of the best purification protocol increasing the yields and folds still crucial and chitin affinity chromatography is much recommended for chitinases purification, however extensive researches and further optimizations were needed for developing of an efficient large scale chitin affinity purification strategy to meet with increased industrial demands.

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REFERENCES

1. Ghasemi, S., G. Ahmadian, N.B. Jelodar, H. Rahimian, S. Ghandili, A. Dehestani and P. Shariati, 2010. "Antifungal chitinases from *Bacillus pumilus* SG2" preliminary report. World Journal of Microbiology and Biotechnology, 26: 1437-1443.
2. Brzezinska, M.S., U. Jankiewicz and M. Walczak, 2013." Biodegradation of chitinous substances and chitinase production by the soil actinomycete *Streptomyces rimosus*; International Biodeterioration and Biodegradation: 84 Special Issue: 104-110.
3. Correa, JU., N. Elango, I. Polacheck and E. Cabib, 1982. "Endochitinase, a mannan-associated enzyme from *Saccharomyces cerevisiae*. J. Biol. Chem., 257: 1392-1397.
4. Lee, Y.G., K.C. Chung, S.G. Wi, J.C. Lee and H.J. Bae, 2009. "Purification and properties of a chitinase from *Penicillium* sp. LYG 0704." Protein Expression and Purification, 65: 244-250.
5. Henrissat, B. and A. Bairoch, 1993. "New families in the classification of glycosyl hydrolases based on amino acid sequence similarities. Biochemical Journal, 293: 781-788.
6. Kurek, D.V., S.A. Lopatin and V.P. Varlamov, 2009. "Prospects of application of the chitin-binding domains to isolation and purification of recombinant proteins by affinity chromatography: a review "Prikladnaia biokhimiia i mikrobiologiia, 45(1): 5-13.
7. Gutiérrez-Román, M.I., M.F. Dunn, R. Tinoco-Valencia and F. Holguín-Meléndez, 2014. "Potentiation of the synergistic activities of chitinases ChiA, ChiB and ChiC from *Serratia marcescens* CFFSUR-B2 by chitobiase (Chb) and chitin binding protein CBP) "World Journal of Microbiology and Biotechnology, 30(1): 33-42.
8. Shimosaka, M., M. Nogawa, X. Wang, M. Kumehara and M. Okazaki, 1995." Production of two chitosanases from a chitosan-assimilating bacterium, *Acinetobacter sp*. strain CHB101". App. Environ. Microbiol., 61(2): 438-442.
9. Guo S.H., J.X. Chen and W.C. Lee, 2004. "Purification and characterization of extracellular chitinase from *Aeromonas schubertii*." Enz. Microb. Technol., 35: 550-556.
10. Wang, S.L. and W.T. Chang, 1997. "Purification and characterization of two bifunctional chitinase/lysozymes extracellularly produced by *Pseudomonas aeruginosa* K-187 in a shrimp and crab shell powder medium." App. Environ. Microbiol., 63(2): 380-386.
11. Chen, H.C., M.F. Hsu and S.T. Jiang, 1997. "Purification and characterization of an exo-N, N-diacetylchitobiohydrolase-like enzyme from *Cellulomonas flavigena* NTOU1." Enz. Microb. Technol., 20: 191-197.
12. Dahiya, N., R. Tewari, R.P. Tewari and G.S. Hoondal, 2005. "Chitinase from *Enterobacter sp*. NRG4: its purification, characterization and reaction pattern." Electronic. J. Boitechnol., 8(2): 134-145.
13. Brurberg, M.B., V.G.H. Eijsink and I.F. Nes, 1994." Characterization of a chitinase gene (Chi a) from *Serratia marcescens* BJL 200 and one-step purification of the gene product." FEMS. Microbiol. Lett., 124: 399-404.
14. Ohishi, K., M. Yamagishi, T. Ohta, M. Suziki, H. Izumida and H. Sano, 1996. "Purification and properties of two chitinases from *Vibrio alginolyticus* H-8." J. Ferment. Bioeng., 82: 598-600.
15. Yamaoka, H., H. Hayashi, S. Karita, T. Kimura, K. Sakka and K. Ohmiya, 1999. "Purification and some properties of chitinase from *Xanthomonas sp*. strain AK." J. Biosc. Bioeng., 88(3): 328-330.
16. Chen, C.T., C.J. Huang, Y.H. Wang and C.Y. Chen, 2004. "Two step purification of *Bacillus circulans* chitinase A1 expressed in *Escherichia coli* periplasm." Prot. Express. Purif., 37: 27-31.
17. Sakai, K., M. Narihara, Y. Kasama, M. Wakayama and M. Moriguchi, 1994. "Purification and characterization of thermostable F-N-acethyl hexosaminidase of *Bacillus stearothermophilus* CH-4 isolated from chitin-containing compost." Appl. Environ. Microbiol., 60(8): 2911-2915.
18. Tsujibo, H., K.K. Minoura, H. Miyamoto, M. Endo, M. Moriwaki and Y. Inamori, 1993. "Purification and properties of a thermostable chitinase from *Streptomyces thermoviolaceus* OPC-520." Appl. Environ. Microbiol., 59(2): 620-622.

19. El-sayed, E., S.M. Ezzat, M.F. Ghaly, M. Mansour and M.A. El-Bohey, 1999. "Purification and characterization of two chitinases from *Streptomyces albovinaceus* S-22." J. Union Arb. Biol., 8(B). Microbiology and Viruses 149-163, 6th International Conference, 8-11 Nov.
20. Pinto, A.D., C.C. Barreto, A. Schrank, C.J. Ulhoa and M.H. Vainstein, 1997. "Purification and characterization of an extracellular chitinase from the entomopathogen *Metarhizium anisopliae*." Can. J. Microbiol., 43: 322-327.
21. Inban, J. and I. Chet, 1991. "Evidence that chitinase produced by *Aeromonas caviae* is involved in the biological control of soil-borne plant pathogens by this bacterium."Soil. Biol. Biochem., 23: 973-978.
22. Fuchs, R.I., S.A. Mc Pherson and D.J. Drahos, 1986. "Cloning of *Serratia marcescens* gene encoding chitinase." Appl. Environ. Microbiol., 51(3): 504-509.
23. Bhushan, B. and G.S. Hoondal, 1998. "Isolation, purification and properties of a thermostable chitinase from an alkalophilic *Bacillus sp.* BG-11." Biotechnol. Lett., 20(2): 157-159.
24. Sakai, K., A. Yokota, H. Kurokawa, M. Wakayama and M. Moriguchi, 1998. "Purification and characterization of three thermostable endochitinases of a noble *Bacillus* strain, MH-1 isolated from chitin-containing compost." App. Environ. Microbiol., 64(9): 3397-3402.
25. Po-Min Kao, Chih-I. Chen, Shu-Chen Huang, Kai-Min Lin, Yung-Chi Chang and Yung-Chuan Liu, 2009. "Preparation of fermentation-processed chitin and its application in chitinase affinity adsorption " Process Biochemistry, 44(3): 343-348.
26. Mukherjee, G. and S.K. Sen, 2006. "Purification, Characterization and Antifungal Activity of Chitinase from *Streptomyces venezuelae* P10." Curr. Microbiol., 53(4): 265-269.
27. Teotia, S., R. Lata and M.N. Gupta, 2004. "Chitosan as a macro affinity ligand and purification of chitinases by affinity precipitation and aqueous two-phase extractions. "J. Chromato. A., 1052: 85-91.
28. Yanai, K., N. Takaya, N. Kojima, H. Horiuchi, A. Ohta and M. Takagi, 1992. "Purification of two chitinases from *Rhizopus oligosporus* and isolation and sequencing of the encoding genes. J. Bacteriol., 174: 7398-7406.